

## IN THE CLAIMS

Please amend the claims as follows.

1. (Currently amended) A recombinant replicon nucleic acid comprising:
  - a) ~~a first~~ nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
  - b) ~~at least one~~ a second nucleic acid sequence encoding an alphavirus nonstructural protein;
  - c) ~~at least one alphavirus subgenomic promoter;~~  
~~at least one IRES element;~~  
~~at least one heterologous nucleic acid~~ an alphavirus subgenomic promoter-IRES-heterologous nucleic acid of interest (NOI) cassette; and
  - d) ~~a third~~ nucleic acid encoding a 3' alphavirus replication recognition sequence.
2. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the ~~second~~ nucleic acid sequence of (b) is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2, nsp3 and nsp4.
3. (Currently amended) The recombinant replicon nucleic acid of claim 1, ~~comprising an original second~~ wherein the nucleic acid sequence of (b) ~~that is~~ a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2 and nsp3 and ~~further comprising wherein the recombinant replicon nucleic acid comprises an additional second~~ a nucleic acid nucleotide sequence encoding alphavirus nonstructural protein nsp4 that is not contiguous with the ~~original second~~ nucleic acid sequence of (b).
4. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the IRES ~~element~~ is selected from the group consisting of cellular IRESs, plant IRESs, mammalian virus IRESs, synthetic IRESs and insect virus IRESs.
5. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the alphavirus subgenomic promoter of (c) is a minimal or modified alphavirus subgenomic

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promoter.

6. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the heterologous ~~nucleic acid~~ NOI of (b) encodes a protein or peptide.

7. (Withdrawn) The recombinant replicon nucleic acid of claim 1, wherein the heterologous ~~nucleic acid~~ NOI is an antisense sequence.

8. (Withdrawn) The recombinant replicon nucleic acid of claim 1, wherein the heterologous ~~nucleic acid~~ NOI encodes a ribozyme.

9. (Currently amended) The recombinant replicon nucleic acid of claim 1, ~~wherein the heterologous nucleic acid encodes~~ further comprising a nucleotide sequence encoding an alphavirus structural protein.

10. (Currently amended) The recombinant replicon nucleic acid of claim 9, wherein the alphavirus structural protein is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

11. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the ~~first~~ nucleic acid sequence ~~encoding a 5' alphavirus replication recognition sequence~~ of (a) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

12. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the ~~second~~ nucleic acid sequence ~~encoding an alphavirus nonstructural protein~~ of (b) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

13. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the alphavirus subgenomic promoter of (c) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

14. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the ~~third~~ nucleic acid sequence encoding a 3' alphavirus replication recognition sequence of (d) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

15. (Withdrawn) The recombinant replicon nucleic acid of claim 1, wherein the IRES ~~element of (c)~~ directs the translation of the gene product encoded by the heterologous ~~nucleic acid NOI of (c)~~, such that at least 80% of the translation of the gene product encoded by the heterologous ~~nucleic acid NOI~~ is controlled by the activity of the IRES ~~element~~.

16. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the IRES ~~elements of (c)~~ directs the translation of the gene product encoded by the heterologous ~~nucleic acid NOI of (c)~~ such that at least 10% of the translation of the gene product encoded by the heterologous ~~nucleic acid NOI~~ is controlled by the activity of the IRES ~~element~~.

17. (Currently amended) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid is RNA.

18. (Withdrawn) The recombinant replicon nucleic acid of claim 1, wherein the nucleic acid is DNA.

19. (Currently amended) ~~The nucleic acid of claim 1, further comprising a spacer nucleic acid sequence located upstream of the IRES element~~ A recombinant replicon nucleic acid comprising:

- a) a nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
- b) a nucleic acid sequence encoding an alphavirus nonstructural protein;
- c) an alphavirus subgenomic promoter-IRES-heterologous nucleic acid of interest (NOI) cassette, said cassette further comprising a spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES; and
- d) a nucleic acid encoding a 3' alphavirus replication recognition sequence.

20. (Currently amended) The recombinant replicon nucleic acid of claim 19, wherein the spacer non-coding nucleic acid sequence is at least 30 nucleotides in length.

21. (Currently amended) The recombinant replicon nucleic acid of claim 19, wherein the spacer non-coding nucleic acid sequence is between 25 and 7500 nucleotides in length.

22. (Currently amended) The recombinant replicon nucleic acid of claim 19, wherein the spacer non-coding nucleic acid sequence is between 150 and 1000 nucleotides in length.

23. (Currently amended) A population of infectious, defective, alphavirus particles, wherein each particle comprises the ~~nucleic acid of claim 19~~ alphavirus particle of claim 27, and the population has no detectable replication-competent virus, as measured by passage on cell culture.

24. (Currently amended) A population of infectious, defective, alphavirus particles, wherein each particle comprises the ~~nucleic acid of claim 19~~ alphavirus particle of claim 28, and the population has no detectable replication-competent virus, as measured by passage on cell culture.

25. (Previously presented) A pharmaceutical composition comprising the population of claim 23 in a pharmaceutically acceptable carrier.

26. (Previously presented) A pharmaceutical composition comprising the population of

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claim 24 in a pharmaceutically acceptable carrier.

27. (Currently amended) An alphavirus particle comprising ~~at~~the recombinant replicon nucleic acid ~~according to~~of claim 1.

28. (Currently amended) An alphavirus particle comprising ~~at~~the recombinant replicon nucleic acid ~~according to~~of claim 19.

29. (Previously presented) The alphavirus particle of claim 27, comprising an attenuating mutation.

30. (Currently amended) The alphavirus particle of claim ~~29~~28, comprising an attenuating mutation.

31. (Currently amended) The recombinant replicon nucleic acid of claim 1, comprising an attenuating mutation.

32. (Currently amended) The recombinant replicon nucleic acid of claim 19, comprising an attenuating mutation.

33. (Currently amended) A population of infectious, defective, alphavirus particles, ~~wherein each particle comprises the nucleic acid of claim 1~~comprising the alphavirus particle of claim 27.

34. (Currently amended) A population of infectious, defective, alphavirus particles, ~~wherein each particle comprises the nucleic acid of claim 19~~comprising the alphavirus particle of claim 28.

35. (Currently amended) A composition comprising the population of claim ~~32~~33, in a

pharmaceutically acceptable carrier.

36. (Currently amended) A composition comprising the population of claim ~~33~~34, in a pharmaceutically acceptable carrier.

37. (Withdrawn) A method of making infectious, defective alphavirus particles, comprising:  
a) introducing into a cell the following:

- (i) ~~athe~~ recombinant replicon nucleic acid ~~according to~~ claim 1, and
- (ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the one or more helper nucleic acids produce all of the alphavirus structural proteins; and

b) producing the alphavirus particles in the cell.

~~37~~38. (Withdrawn) The method of claim ~~36~~37, wherein the recombinant replicon nucleic acid further comprises ~~at least one heterologous nucleic acid encoding a nucleotide sequence encoding~~ an alphavirus structural protein.

~~38~~39. (Withdrawn) The method of claim ~~36~~37, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, a nucleic acid encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

~~39~~40. (Withdrawn) The method of claim ~~36~~37, wherein the helper nucleic acid is a recombinant nucleic acid comprising a promoter and nucleotide sequences encoding one or more alphavirus structural proteins.

~~40~~41. (Withdrawn) The method of claim ~~38~~40, wherein the helper nucleic acid is DNA.

4142. (Withdrawn) The method of claim 4041, wherein the promoter is a CMV promoter.

4243. (Withdrawn) The method of claim 4041, wherein the helper nucleic acid comprises nucleotide sequences encoding all of the alphavirus structural proteins.

4344. (Withdrawn) The method of claim 3637, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an IRES element, a ~~nucleic acid~~ nucleotide sequence encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

4445. (Canceled).

4546. (Withdrawn) A method of making infectious, defective alphavirus particles, comprising:  
a) introducing into a cell the following:

i) an alphavirus replicon RNA comprising a 5' alphavirus replication recognition sequence, nucleic acid sequence(s) encoding alphavirus nonstructural proteins, an alphavirus subgenomic promoter, a heterologous nucleic acid sequence and a 3' alphavirus replication recognition sequence; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise the recombinant nucleic acid of claim 48 ~~comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, an IRES element, a nucleic acid encoding one or more alphavirus structural proteins, downstream of the IRES element and a 3' alphavirus replication recognition sequence~~, whereby all of the alphavirus structural proteins are produced in the cell; and

b) producing the alphavirus particles in the cell.

4647. (Withdrawn) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

i) ~~an alphavirus replicon RNA comprising a 5' alphavirus replication recognition sequence, nucleic acid sequence(s) encoding alphavirus nonstructural proteins, at least one alphavirus subgenomic promoter, at least one IRES element, at least one heterologous nucleic acid sequence and a 3' alphavirus replication recognition sequence~~the recombinant replicon RNA of claim 1; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise a recombinant nucleic acid comprising:

a) a 5' alphavirus replication recognition sequence;

b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, wherein the NOI encodes one or more alphavirus structural proteins;

c) and a 3' alphavirus replication recognition sequence comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, an IRES element, a nucleic acid encoding one or more alphavirus structural proteins, downstream of the IRES element and a 3' alphavirus replication recognition sequence, whereby all of the alphavirus structural proteins are produced in the cell; and

b) producing the alphavirus particles in the cell.

4748. (Currently amended) A recombinant nucleic acid comprising:

a) a 5' alphavirus replication recognition sequence;

b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, wherein the NOI encodes an alphavirus subgenomic promoter, an IRES element, a nucleic acid encoding one or more alphavirus structural proteins; and

c) a 3' alphavirus replication recognition sequence.

4849. (Currently amended) A cell comprising the recombinant nucleic acid of claim 4748.

4950. (Currently amended) The recombinant replicon nucleic acid of claim 1, further comprising an alphavirus packaging signal.



~~5051.~~ (Currently amended) The recombinant replicon nucleic acid of claim ~~41~~19, further comprising a ~~spacer nucleic acid sequence upstream of an IRES element~~an alphavirus packaging signal.

~~5152.~~ (Currently amended) The ~~nucleic acid of claim 47, further comprising a spacer nucleic acid sequence upstream of an IRES element~~A recombinant nucleic acid comprising: a) a 5' alphavirus replication recognition sequence;  
b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, said cassette further comprising a spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES, wherein the NOI encodes one or more alphavirus structural proteins;  
c) and a 3' alphavirus replication recognition sequence.

~~5253.~~ (Withdrawn) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim 23.

~~5354.~~ (Withdrawn) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the population of claim 24.

~~5455.~~ (Withdrawn) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the ~~population~~composition of claim ~~33~~25.

~~5556.~~ (Withdrawn) A method of eliciting an immune response in a subject, comprising administering to the subject an immunogenic amount of the ~~population~~composition of claim ~~34~~26.

~~5657.~~ (Canceled).

~~5758.~~ (Currently amended) A recombinant replicon nucleic acid comprising:

- ~~a) a first~~ nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
- ~~b) at least one~~ a second nucleic acid sequence encoding an alphavirus nonstructural protein;
- ~~c) a first alphavirus subgenomic promoter;~~  
a first IRES element, downstream of the first alphavirus subgenomic promoter of (c);  
~~a first heterologous nucleic acid~~ alphavirus subgenomic promoter-IRES-heterologous NOI cassette;
- ~~d) a second alphavirus subgenomic promoter;~~  
~~a second IRES element, downstream of the second alphavirus subgenomic promoter-IRES-~~  
heterologous NOI cassette; and
- ~~e) a third~~ nucleic acid encoding a 3' alphavirus replication recognition sequence.

~~5859.~~ (Currently amended) The recombinant replicon nucleic acid of claim ~~5758~~, further comprising an alphavirus packaging signal.

~~5960.~~ (Canceled).

61. (New) A recombinant replicon nucleic acid comprising:

- a) a nucleic acid sequence encoding a 5' alphavirus replication recognition sequence;
- b) a nucleic acid sequence encoding an alphavirus nonstructural protein;
- c) a first alphavirus subgenomic promoter-IRES-heterologous NOI cassette, said cassette further comprising a first spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES;
- d) a second alphavirus subgenomic promoter-IRES-heterologous NOI cassette, said cassette further comprising a first spacer non-coding nucleic acid 3' to the alphavirus subgenomic promoter and 5' to the IRES; and
- e) a nucleic acid encoding a 3' alphavirus replication recognition sequence.

62. (New) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid

sequence of (b) is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2, nsp3 and nsp4.

63. (New) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (b) is a contiguous nucleotide sequence encoding alphavirus nonstructural proteins nsp1, nsp2 and nsp3 and wherein the recombinant replicon nucleic acid comprises a nucleotide sequence encoding alphavirus nonstructural protein nsp4 that is not contiguous with the nucleic acid sequence of (b).

64. (New) The recombinant replicon nucleic acid of claim 19, wherein the IRES is selected from the group consisting of cellular IRESs, plant IRESs, mammalian virus IRESs, synthetic IRESs and insect virus IRESs.

65. (New) The recombinant replicon nucleic acid of claim 19, wherein the alphavirus subgenomic promoter of (c) is a minimal or modified alphavirus subgenomic promoter.

66. (New) The recombinant replicon nucleic acid of claim 19, wherein the heterologous NOI of (b) encodes a protein or peptide.

67. (New) The recombinant replicon nucleic acid of claim 19, wherein the heterologous NOI is an antisense sequence.

68. (New) The recombinant replicon nucleic acid of claim 19, wherein the heterologous NOI encodes a ribozyme.

69. (New) The recombinant replicon nucleic acid of claim 19, further comprising a nucleotide sequence encoding an alphavirus structural protein.

70. (New) The recombinant replicon nucleic acid of claim 69, wherein the alphavirus structural

protein is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

71. (New) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (a) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

72. (New) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (b) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

73. (New) The recombinant replicon nucleic acid of claim 19, wherein the alphavirus subgenomic promoter of (c) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

74. (New) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid sequence of (d) is from an alphavirus selected from the group consisting of Sindbis virus, SFV, VEE, S.A. AR86 virus, Ross River virus, EEE and WEE.

75. (New) The recombinant replicon nucleic acid of claim 19, wherein the IRES of (c) directs the translation of the gene product encoded by the heterologous NOI of (c), such that at least 80% of the translation of the gene product encoded by the heterologous NOI is controlled by the activity of the IRES.

76. (New) The recombinant replicon nucleic acid of claim 19, wherein the IRES of (c) directs the translation of the gene product encoded by the heterologous NOI of (c) such that at least 10% of the translation of the gene product encoded by the heterologous NOI is controlled by the activity of the IRES.

77. (New) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid is RNA.

78. (New) The recombinant replicon nucleic acid of claim 19, wherein the nucleic acid is DNA.

79. (New) A cell comprising the recombinant replicon nucleic acid of claim 19.

80. (New) A cell comprising the recombinant replicon nucleic acid of claim 1.

81. (New) A cell comprising the recombinant nucleic acid of claim 52.

82. (New) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

(i) the recombinant replicon nucleic acid of claim 19, and

(ii) one or more helper nucleic acids encoding alphavirus structural proteins,

wherein the one or more helper nucleic acids produce all of the alphavirus structural proteins; and

b) producing the alphavirus particles in the cell.

83. (New) The method of claim 82, wherein the recombinant replicon nucleic acid further comprises a nucleotide sequence encoding an alphavirus structural protein.

84. (New) The method of claim 82, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an alphavirus subgenomic promoter, a nucleic acid encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

85. (New) The method of claim 82, wherein the helper nucleic acid is a recombinant nucleic acid comprising a promoter and nucleotide sequences encoding one or more alphavirus structural

proteins.

86. (New) The method of claim 85, wherein the helper nucleic acid is DNA.

87. (New) The method of claim 85, wherein the promoter is a CMV promoter.

88. (New) The method of claim 85, wherein the helper nucleic acid comprises nucleotide sequences encoding all of the alphavirus structural proteins.

89. (New) The method of claim 82, wherein the helper nucleic acid is a recombinant nucleic acid comprising a 5' alphavirus replication recognition sequence, an IRES element, a nucleotide sequence encoding an alphavirus structural protein and a 3' alphavirus replication recognition sequence.

90. (New) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

i) an alphavirus replicon RNA comprising a 5' alphavirus replication recognition sequence, nucleic acid sequence(s) encoding alphavirus nonstructural proteins, an alphavirus subgenomic promoter, a heterologous nucleic acid sequence and a 3' alphavirus replication recognition sequence; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise the recombinant nucleic acid of claim 52, whereby all of the alphavirus structural proteins are produced in the cell; and

b) producing the alphavirus particles in the cell.

91. (New) A method of making infectious, defective alphavirus particles, comprising:

a) introducing into a cell the following:

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i) the recombinant replicon RNA of claim 19; and

ii) one or more helper nucleic acids encoding alphavirus structural proteins, wherein the helper nucleic acid(s) comprise a recombinant nucleic acid comprising:

a) a 5' alphavirus replication recognition sequence;

b) an alphavirus subgenomic promoter-IRES-heterologous NOI cassette, wherein the NOI encodes- one or more alphavirus structural proteins;

c) and a 3' alphavirus replication recognition sequence, whereby all of the alphavirus structural proteins are produced in the cell; and

b) producing the alphavirus particles in the cell.

92. (New) The recombinant nucleic acid of claim 6, wherein the peptide is an immunogen.

93. (New) The recombinant nucleic acid of claim 66, wherein the peptide is an immunogen.